

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Masao MATSUOKA et al.

Application No.: 10/667,359

Filed: September 23, 2003

For. METHOD TO PROTECT TRANSGENES FROM SILENCING

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

SIr:

- 1. We, Maseo MATSUOKA and Koji AKASAKA, declare the following:
- 2. Masao MATSUOKA is a citizen of Japan, and has the following mailing address: <u>Doginosato 4-13-5-204</u>, Otsu 520-0246, Jt PAN.
- 3. Koji AKASAKA Is a citizen of Japan, and has the following mailing address: Uchara 3-12-15-202, Shibuya-ku, Tokyo 157-0064: JAPAN
- 4. The subject matter of Hino et al., Blood 2002, Vol. 100, No. 11, Abstract No. 5522 (Hino et al.) was derived from our own inventive work;
- 5. Shinjiro HINO, Pan JUN, and Shuhei TAGUWA are merely co-authors of Hino et al. and did not have a role in inventing the subject matter of Hino et al.;
- Shinjiro HINO, Pan JUN, and Shuhel TAGUWA were working as graduate students under the direction and supervision of Masao MATSUOKA;
- 7. We are the only inventors of the Invention disclosed and claimed in the above-identified application;

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- 8. Shinjiro HINO, Pan JUN, and Shuhel TAGUWA are not co-inventors of the invention disclosed and dalmed in the above-identified application;
- We further declare that all statements made herein of our own knowledge are true and that all statements on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Oct 24, 2006

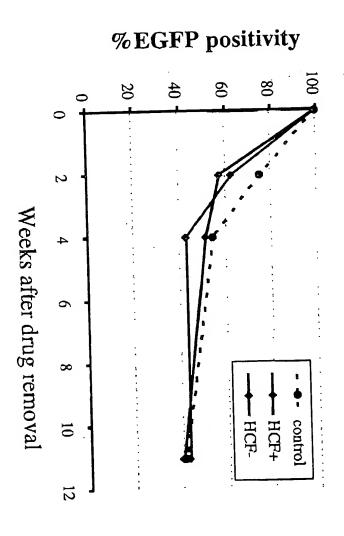
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Date: Oct. 20, 2006

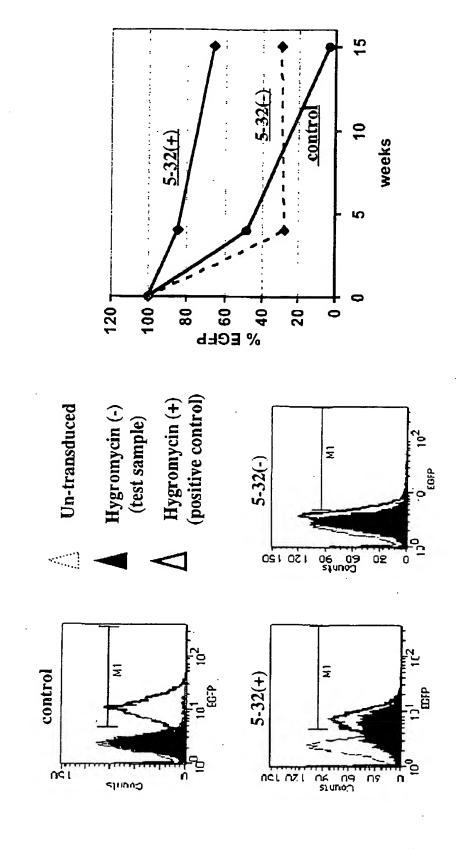
KOII AKASAKA



HCF1, which showed enhancer blocking activity, does not show anti-silencing effect



Human DNA fragment, 5-32, showed both enhancer blocking activityand anti-silencing effect



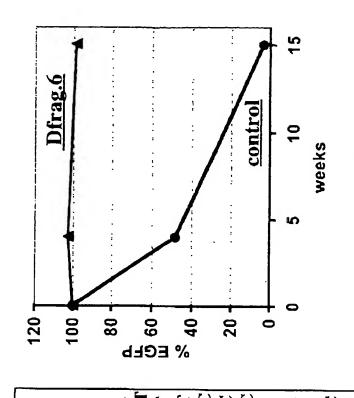
Anti-silencing effect by deleted Ars insulator

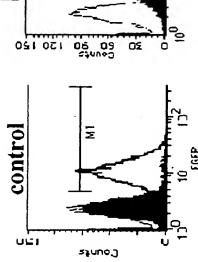
Fragment 6-deleted Ars

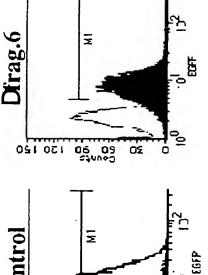
Enhancer blocking activity diminished by deletion of fragment 6.

AAAATGCTTTTCCAAAAGAATGGGGGGGGGGGC TGACATGTAAGCATCTCAAGAAGCATATTTCTTGC **GGCGTTTAATTACGGTCAGATGAGCAATTTTTGAT** TGGACGCTCCCGAACCCCGACGTTCCGCGGGCCC CATTCTTCTTGCTCTTGCTTTCTATTTCTCTTT **ACCTTAATTTGTTGACACTGGTAGTTAACTATT** GGGAGCAGAACCCCTGTAAGCTCAGGGGTTTTTA

AAGTTATGTAATTTCAGCATTATGTGTAAACCACC **GTTATGGAATAAGAATAAACCACATTTCAATTTA** (GGCTGTTAATTTACAAACGCATAAAAAAAATATA **ACCITICITICICITITCCCTIGCICICCTTA AGTGCCCCCCCCCCCCCCTCCCGATCTACACT** CCCTTTCCCTTCCCCACCCTTCTCCACACTTGTT TTTCCCCCGAGCCCCCCCCCGATCAATACGCC TTGTTGGCGGAAAAATCGCAACTGATCTCCCCT **ATITACTAAAGAATGAGGAAAAATCTCGGG** GGCGGGATTACCTGCAAATTATC







Relationship between anti-silencing effect and enhancer blocking effect

Anti-silencing	+	!	+
Enhancer blocking		+	+
	Ars A6	HCF1	5-32